REMARKS

The Office Action of February 27, 2009, has been received and reviewed. Reconsideration is respectfully requested in view of the remarks presented herein.

Rejections under 35 U.S.C. § 103(a)

Claims 10-14, 19-23, 28-32 stand rejected under 35 U.S.C. § 103(a) as assertedly being obvious over Nagahori *et al.* (Chem. BioChem. (2002) vol. 3, p. 836-844) (hereinafter "Nagahori") in view of Silverman ((1992) Drug Discovery, Design, and Development in "The Organic Chemistry of Drug Design and Drug Action." Published by Academic Press, p.4, 16 and 17) (hereinafter "Silverman") (collectively hereinafter "the references"). Applicants respectfully traverse the rejections as hereinafter set forth.

To establish a *prima facie* case of obviousness, the prior art itself or "the inferences and creative steps that a person of ordinary skill in the art would [have] employ[ed]" at the time of the invention are to have taught or suggested the claim elements. Additionally, the Examiner must determine whether there is "an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1740-1741, 167 L.Ed.2d 705, 75 USLW 4289, 82 U.S.P.Q.2d 1385 (2007). Further, rejections on obviousness grounds "cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *Id* at 1741, quoting *In re Kahn*, 441, F.3d 977, 988 (Fed. Cir. 2006). "Often, it will be necessary for a [fact finder] to look to interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed . . . . To facilitate review, this analysis should be made explicit." *Id.* Furthermore, to establish a *prima facie* case of obviousness there must have been a reasonable expectation of success. M.P.E.P. § 2143.02. Underlying the obvious determination is the fact that statutorily prohibited hindsight cannot be used. *KSR*, 127 S.Ct. at 1742.

Applicants respectfully submit that the references cannot make obvious claims 10-14, 19-

23, and 28-32, absent applicants' disclosure, as no motivation would have existed to modify the references to arrive at the present claims, the references teach away from the present claims, and no reasonable expectation of success would have existed as of the priority date of the instant application.

Lack of Motivation to Modify the Compounds of Nagahori as Taught by Silverman

One of ordinary skill in the art would not have been motivated to modify the mannose containing molecules taught by Nagahori as taught by Silverman. As is clear from Nagahori, the taught compositions function by binding of the pilus of the bacteria to prevent association of the bacteria with the epithelium of the host. Nagahori at page 836, ¶ 2, and page 840, first paragraph of Discussion, as well as the present Specification as published at ¶¶ [0009], [0044], and [0054]-[0060]. Taking the purported teachings of Silverman at face value (which applicants dispute *infra*), Silverman teaches that the elongation of saturate carbon side-chains to 5-9 atoms "corresponds to increased lipophilicty of the molecule, which permits penetration into cell membranes." Silverman at page 16. However, in attempting to bind the pilus of free circulating bacteria, one of ordinary skill in the art would have wished to avoid penetration of the cell membrane as the compounds would be ineffective in treating an infection of Gram-negative bacterium if such compounds were localized within cells (where they would be unavailable for binding the pilli of the bacteria).

Moreover, one of ordinary skill in the art would have concluded that the increased localization of a compound with a aliphatic chain length of between 5 and 9 with the cell membrane would increase localization of bacteria that bind the compound with the epithelium. Increased association with and penetration into cell membranes is exactly the type of interaction one of ordinary skill in the art would have wished to avoid in preventing association with the epithelium.

For at least the foregoing reason, applicants respectfully assert that one of ordinary skill in the art would have found no motivation to modify the compounds of Nagahori as taught be Silverman as suggested in the Office Action.

The References Teach Away From the Present Claims

Applicants note that each of the claims under consideration recites a method using a mannose having a single mannose residue. Applicants submit that the references teach away from using single mannose compositions. Specifically, Nagahori teaches that "[t]wo important factors that strongly influence the affinity of *E. coli* adhesin are: . . . 2) the presence of multiple mannosyl residues that can span a distance of 20 nm or longer on a relatively inflexible structure." Nagahori, abstract. Thus, Nagahori teaches away from the using the mannose compounds recited by the presently claimed methods.

As Silverman is directed to optimization of aliphatic chain length, it does not contradict the explicit teaching of Nagahori regarding the use of compounds having multiple mannosyl residues. Consequently, one of ordinary skill in the art, considering the teachings of the references as a whole, would not have used single mannose residue molecules as presently claimed, but would instead have focused on oligomannose molecules. Thus, the references teach away from the present claims.

Other researchers confirm Nagahori's teachings with respect to multiple mannose residues. For example, Firon et al. (1984), at page 1090 teach that "[i]n general, it appears that mannose-specific bacteria preferentially bind structures found in short oligomannose chains of *N*-glycosyl-linked glycoproteins" (Infection and Immunity, Mar. 1984, 1088-1090) (previously submitted). Further, Firon et al. (1983) (also previously submitted), at page 235, teaches that "[i]t appears that the binding sites of the three bacterial lectins tested exhibit preference for structures found in N-glycosylic oligomannoside units of mammalian cell surface glycoproteins."

In view of at least the foregoing, one of ordinary skill in the art would have concluded that multiple mannosyl residues were needed for the efficient binding of the bacterial pilus. Thus, the references and the art teach away from the use of single mannose residue compounds.

No Reasonable Expectation of Success

The Office relies upon Silverman for the suggestion that "lengthening of a saturated carbon die chain from one (methyl) to five to nine carbon atoms (pentyl to nonyl) produces an increase in pharmacological effects." Office Action at page 6. However, the art does not support

increase in pharmacological effects." Office Action at page 6. However, the art does not support the apparent certainty of this suggestion. For example, Eley and Triumalashetty (AAPs PharmsciTech 2001 2(3) article 9 (submitted herewith)), at page 6, teach that "[i]t seems unlikely that a relationship exists between the structures of these alkylglycosides and their effects. Activity appears unrelated to the carbon chain length or the carbohydrate moiety of the alkyglycoside" (emphasis added). Thus, one or ordinary skill in the art, when considering the teachings Ely and Triumalashetty, would have concluded that there would have been no no reasonable expectation of success for increasing a pharmacological effect when lengthening a side chain of any and all potential pharmaceutics.

Furthermore, Silverman's statement that "for many series of compounds, lengthening of a saturated carbon side chain from one (methyl) to five to nine atoms (pentyl to nonyl) produces an increase in pharmacological effects" is very general and such a statement would not have been used by one of ordinary skill in the art as a standard for the rational design of more effective putative drug compounds in each and every instance.

To illustrate this, the Examiner is referred to Firon et al. (1983) describing a difference in carbohydrate specificity of the mannose-binding FimH adhesins (lectins) of the enteric bacteria possessing type 1 fimbriae, in particular *Escherichia coli, Klebsiella pneumonia, and Salmonella typhimurium*. Therein, the relative inhibitory activity of various D-mannose derivates on agglutination of yeast cells or guinea pig erythrocytes was measured to gain insight into the specificity and structure of the combining sites of the FimH lectins.

In the abstract, it is stated that "the combining site of the *Klebsiella pneumonia* fimbrial lectin is probably similar to that of *E. coli*, but that of the *Salmonella typhimurium* fimbrial lectin differs considerably. It appears that the combining sites of the three bacterial lectins tested exhibit preference for structures found in N-glycosydic oligomannoside units of mammalian cell surface glycoproteins."

Based on the differences in inhibitory activities (see Table II, e.g., compound 6, amongst others), it is suggested that the *E. coli* and *K. pneumonia* lectins might possess a hydrophobic region in the combining site or close to it, which is likely not the case for *S. typhymurium*. Thus, one of ordinary skill in the art is left in doubt as to the best strategy to follow for designing more

effective inhibitory compounds, particularly since differences exist between otherwise related D-mannose-specific enteric bacterial strains possessing type 1 fimbriae.

Similar conclusions are to be drawn from a later study by Firon et al. (1984), where again inhibitory potencies of various mannose derivatives on agglutination of yeast cells by different enterobacteria are compared (see Table 2, see, e.g., compound 3, amongst others). Also here, it is concluded that although classified under the general term mannose specific (or mannose sensitive), the fimbrial lectins of different genera and species exhibit differences in sugar specificities. Consequently, one of ordinary skill in the art would conclude the there is little reasonable expectation of success in using standard approaches such as the one of Silverman, as the publications of Firon et al., suggest that the broad applicability of the standard approaches are unlikely to work in many cases.

In view of at least the foregoing, applicants respectfully request withdrawal of the rejections under 35 U.S.C. §103(a).

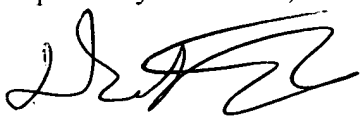
Rejoinder

Applicants respectfully request rejoinder of claims 15-18, 24-27, and 33-36 as claims 10, 19, and 28 are generic for these claims. Further, if claims require all the elements of an allowable claim, those claims will be eligible for rejoinder. M.P.E.P. § 821.04; *see also In re Ochiai*, 71 F.3d 1565 (Fed. Cir. 1995); *In re Brouwer*, 77 F.3d 422 (Fed. Cir. 1996). Applicants respectfully submit that each of claims 15-18, 24-27, and 33-36 incorporate all of the elements of one of claims 10, 19, and 28. Applicants believe claims 10, 19, and 28 are in condition for allowance. As such, applicants respectfully request the rejoinder of claims 15-18, 24-27, and 33-36 which each include all the elements of one of allowable claims 10, 19, and 28.

CONCLUSION

In light of the foregoing remarks, applicants respectfully request allowance of the application. If questions remain after consideration of the foregoing, or if the Office should determine that there are additional issues which might be resolved by a telephone conference, the Office is kindly requested to contact applicants' attorney at the address or telephone number given herein.

Respectfully submitted,



Daniel J. Morath, Ph.D.
Registration No. 55,896
Attorney for Applicants
TRASKBRITT, P.C.
P.O. Box 2550
Salt Lake City, Utah 84110-2550
Telephone: 801-532-1922

Date: May 26, 2009

Enclosure: IDS

AAPS PharmSciTech

AAPS PharmsciTech 2001; 2 (3) article 19
(http://www.pharmscitech.com).

In Vitro Assessment of Alkylglycosides as Permeability Enhancers

John G. Eley[1]* and Prasanth Triumalashetty[1]

[1]Department of Pharmaceutical Sciences, Harrison School of Pharmacy, 302 Walker Building, Auburn University, Auburn, AL 36849

Submitted: February 27, 2001; Accepted: September 30, 2001; Published: October 19, 2001.

ABSTRACT

A series of alkylglycosides has been evaluated on human cell lines to determine its ability to open cellular tight junctions. Alkylglycosides were applied to cell monolayers; the resulting change in resistance was determined by transepithelial electrical resistance measurements. Change in resistance across cell monolayers is an indication of tight junction activation, whereas subsequent increase in resistance signifies monolayer recovery. Of the 13 alkylglycosides tested, 4 caused irreversible solubilization of cell membranes, 5 allowed a partial recovery of the monolayer after a relatively rapid reduction in resistance, and 4 induced a decrease in resistance with more complete cell recovery. Alkylglycosides allowing extensive cell recovery after removal may indicate tight junctions' activity dominance over membrane fluidity. Repeated application of alkylglycosides for 6 hours lowered resistance across cells, which returned to near-normal values after a recovery period of 48 hours. A model dye was transported across the cell monolayer only in the presence of an alkylglycoside, although recovery of cells was incomplete. Activity of the alkylglycosides was unrelated to either the carbon chain length or to the carbohydrate moiety. A direct correlation was established between the concentration of applied alkylglycoside and reduction in resistance over a constant time period. Dodecylmaltoside and octylglucoside were found to be optimal in decreasing resistance at low concentrations and allowing significant recovery of cells. Therefore these 2 alkylglycosides may be useful in facilitating drug transport across biological membranes.

KEYWORDS: permeability/enhancers, alkylglycosides, tight junctions.

***Corresponding Author:** John G. Eley, Department of Pharmaceutical Sciences, Harrison School of Pharmacy, 302 Walker Building, Auburn University, Auburn, AL 36849; Telephone:. (334-) 844-8303; Facsimile: (334-) 844-8331; E-mail: eleyjohn@auburn.edu

INTRODUCTION

Delivery of protein and peptide drugs, including some of the recently introduced biotechnology drugs, must usually be accomplished through the parenteral route. Other potential routes have been the targets in formulation research for some years; however, transport across mucous membranes usually results in low bioavailability. Permeability-enhancing agents may be useful in facilitating transport of these problematic drugs across biological membranes.

In one approach to finding a suitable permeability candidate as an adjuvant, various administration routes were investigated for insulin [1-4], using formulations containing surfactants or bile salts as permeability enhancers. It was found that such agents required relatively high concentrations for successful cell membrane disruption. In other studies, the effects of nonionic surfactants—polysorbates 20, 60, and 85; cholesteryl polyoxyethylene ether; and lanolin-based polyoxyethylene ether—were observed on Caco-2 cell monolayers. Although the agents were active as absorption enhancers, increased permeability resulted from solubilization of membrane and membrane components [5]. An intestinal perfusion model showed that a variety of agents, including sodium dodecyl sulfate and nonylphenoxpolyoxy-ethylene, were effective in aiding the transport of the polar agent phenol red. However, these agents caused intestinal wall damage in situ [6].

Clinical trials using a formulation of insulin plus deoxycholate, glycocholate, or taurocholate indicated that such agents need to be administered in high concentrations, causing local irritation that implies cell toxicity [7,8].

Dodecylmaltoside, an alkylglycoside, has been successful in mediating nasal and ocular absorption of LysPro insulin to rats [9]. The low concentrations required to enhance the delivery of insulin [10] suggested that there might be other candidates within

the alkylglycoside series suitable for clinical trials. This project is therefore concerned with the investigation of alkylglycosides as permeability enhancers at a more basic level of evaluation using cell culture models.

Under ideal conditions, a permeability enhancer would activate intercellular tight junctions , allowing a drug to pass through without damage to cells, therefore allowing them a timely recovery. The hypothesis proposed was that suitable alkylglycosides would transiently activate opening of tight junctions when used at low concentrations, subsequently allowing them to close after removal, leading to a rapid recovery of cells. This would suggest that paracellular transport was dominant and cell membranes were not solubilized to a degree that caused damage. The aim of this investigation was to evaluate a series of alkylglycosides by using 2 cell culture models that record the change in resistance across cell monolayers. Alkylglycosides consist of a series of nonionic surfactants of alkyl chain lengths of between 5 and 13 carbons attached to either a monosaccharide or disaccharide of a molecular weight between 264 and 538 [11] (Table 1).

Table 1. A Comparison of the Alkylglycosides Used Listed by Increasing Carbon Chain Length - *Carbon chain length. †Carbohydrate. ‡Final concentration in mM used to lower transepithelial electrical resistance across cell monolayers.

Alkylglycoside	Carbon Chain Length*	Carbohydrate† G = Glucose M = Maltose	Final Concentration (mM)‡
Hexylmaltoside	5	M	0.023
Hexylglucoside	5	G	0.37
Heptylglucoside	6	G	0.36
Octylmaltoside	7	M	0.22
Octylglucoside	7	G	0.34
Nonylmaltoside	8	M	0.021
Nonylglucoside	8	G	0.032
Decylmaltoside	9	M	0.20
Decylglucoside	9	G	0.31
Dodecylmaltoside	11	M	0.19
Dodecylglucoside	11	G	0.28
Tridecylmaltoside	12	M	0.19
Tetradecylmaltoside	13	M	0.018

Transepithelial electrical resistance (TEER) measurements were used to monitor activity of tight junctions. An alkylglycoside used at low concentrations, able to produce rapid onset of action in opening tight junctions and a short cell recovery time upon removal with no cell damage, may be a suitable candidate for an adjuvant.

MATERIALS AND METHODS

Materials

Alkylglycosides hexylglucoside (c = 5), hexylmaltoside (c = 5), heptylglucoside (c = 6), octylglucoside (c = 7), octylmaltoside (c = 7), nonylglucoside (c = 8), nonylmaltoside (c = 8), decylglucoside (c = 9), decylmaltoside (c = 9), dodecylmaltoside (c = 9), and tetradecylmaltoside (c = 13) were purchased from Sigma (St Louis, MO); dodecylglucoside (c = 11) and tridecylmaltoside (c = 12) were purchased from Anatrace (Maumee, OH). "C" is equal to the number of carbon atoms in the alkyl chain. HT-29 Cl.19A cells were obtained from Dr C.L. Laboisse, Paris, France. T-84 cells were purchased from the American Type Culture Collection (Manassas, VA).

All cell culture materials, Hank's balanced salt solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM)/HAM's F-12, Eagle's Minimum Essential Medium/Earle's Balanced Salt Solution, heat inactivated fetal bovine serum (FBS), nonessential amino acids (NEAA), trypsin/EDTA solution, and phosphate buffered saline were purchased from HyClone (Logan, UT). Gentamicin was purchased from Sigma (St Louis, MO). Cell flasks, pipettes, and general cell consumables were purchased from Fisher Scientific (Atlanta, GA). Transwell permeable support (cell culture inserts, clear polyester membranes) was purchased from Corning Scientific Products (Acton, MA). All other chemicals and materials were of analytical grade and obtained from Fisher Scientific (Atlanta, GA).

Cell Culture Conditions

T-84 cells were obtained at passage number 53 and HT-29 Cl.19A subclone of HT-29 at passage number 27. Cells were seeded at 1 10^4 cells/mL in 75cm^2 flasks until confluent. T-84 cells were maintained in DMEM/Ham's F-12 supplemented with 5% heat inactivated FBS and HT-29 Cl.19A cells in DMEM low glucose with 10% FBS. All media contained

NEAA and 0.01% gentamicin. Cells were maintained at 37°C in an atmosphere of 5% CO_2.

Cells were passaged by rinsing with calcium/magnesium-free HBSS, then adding 0.25% trypsin/1 mM EDTA for 1 to 1.5 minutes. After aspiration, cells were placed in an incubator at 37°C for 15 minutes, after which medium was added to detach the monolayer. Cells were reseeded in new flasks at 1 10^4 cells/mL. These cells were passaged approximately once every 5 days depending on their advancement in the log-phase of growth. Medium was changed every 2 to 3 days. Cells were transferred to permeable polyester filters (surface area 1 cm^2 or 4.7 cm^2) within Transwell plates by harvesting from 75 cm^2 flasks and seeding at 7.5 10^4 cells/cm^2 after counting on a Coulter Counter Mode Z1 (Miami, FL.)

Monolayer Integrity

Resistance across cell monolayers was measured every 24 hours by TEER after the cells had become confluent. The measuring instrumentation consisted of an Evom Epithelial voltohmmeter and an Endohm tissue resistance measurement chamber (World Precision Instruments, Sarasota, FL). The voltohmmeter attached to the chamber (capable of accommodation a 6-, 12-, or 24-mm diameter well) provided reproducible resistance measurements of cell monolayers in culture wells. Wells containing cells were transferred to the Endohm chamber and a cap placed in position. Both chamber and cap contained a pair of concentric electrodes consisting of a voltage-sensing silver/silver pellet in the center, plus an annular current electrode. The height of the top electrode is adjustable to fit cell culture wells to the correct depth and allow a uniform current density to flow across the well membrane and cell monolayer. Because of the uniform density of the alternating current (AC) square wave, current from the voltohmmeter or membrane capacitance was largely eliminated. High resistance measurements (this means a high-resistance measurement.) indicated that cells were closely packed and forming tight junctions. Blank calibration wells were used to determine background resistance. Resistance of cell monolayers was determined as resistance minus backgrounds multiplied by the surface area of the insert in ohm cm^2. HT-29Cl.19A and T-84 cells were used at resistance readings of between 800 and 1000 ohms cm^2.

Addition of Alkylglycosides

Cells were maintained in serum-free medium for 24 hours before adding alkylglycosides. All experiments were conducted at 37°C, and cells were allowed to attain a steady resistance reading before alkylglycosides, dissolved in medium, were applied. This stock solution (1%) was added to the apical cell medium to give accumulated final concentrations. In the first set of experiments, additions were made to give final concentration from 0.001% to 1% added at 5-minute intervals and washed after 20 minutes. The maximum concentration of alkylglycoside used during recovery time trials did not exceed 0.1%. Further addition ceased when the resistance across cell monolayers decreased by at least 80% to 90%, as recorded by TEER measurements. Cells were washed twice with HBSS, fresh medium added, and allowed to recover at 37°C. For the time course effects on cells alkylglycosides were added at final concentrations of 0.01%, 0.05%, 0.10%, 0.50%, and 1.0% at 0, 5, 10, 15, and 20 minutes when resistance was recorded.

Repeated Addition of Alkylglycosides

Cells were exposed to dodecylmaltoside and octylglucoside (0.1%) for a 20-minute period every 2 hours for a total of 6 hours. After each application, cells were washed with HBSS and monitored for recovery by TEER for 48 hours. Time to cell recovery was measured after the final application of alkylglycoside had been removed.

Transport of Model Dye

Brilliant blue (0.25 mg/mL) was added to T-84 cell monolayers in the absence and presence of dodecyglucoside and octylglucoside (0.06%). Resistance across the cell monolayer was recorded by TEER. After addition of alkylglycosides and dye, the cells were kept at 37°C. Samples (1 mL) were taken under sink conditions at 2, 5, 10, 15, 20, 30, and 45 minutes. Samples were diluted with 5 mL HBSS and analyzed for dye on a Beckman DU-65 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA.) at 630 nm. Cells were washed with HBSS and incubated for recovery.

RESULTS

The effective concentration of alkylglycosides required to open tight junctions with minimal disruption of membrane components was between 0.01% and 0.1%.

Above this concentration, cells showed little evidence of recovery. When hexylmaltoside, nonylmaltoside, nonylglucoside, and tetradecylmaltoside were each applied to T-84 and HT-29Cl19A cells, they lowered resistance by more than 80% in 8 minutes at 0.01%. These agents were not investigated further because after removal of alkylglycoside no significant increase in resistance was observed over a period of 18 hours, indicating no recovery of cells (Table 2).

Decylmaltoside, tridecylmaltoside, hexylglucoside, decylglucoside, and dodecylglucoside showed no significant effect at 0.01%. At 0.1%, resistance was lowered by 90% in 12 minutes. Only partial recovery of cells occurred within 18 hours (Table 2), which was not deemed significant enough for further investigation.

After applying heptylglucoside, octylglucoside, octylmaltoside, and dodecylmaltoside T-84 cells (Figure 1) and HT-29Cl19A cells (Figure 2) for 20 minutes at 0.1%, resistance was lowered resistance over a range of 7% to 95%. There was considerable cell recovery within 18 hours of alkylglycoside removal (Table 2). Dodecylmaltoside lowered resistance by 95% with a recovery of 90% on T-84 cells and by 88% with a recovery of 90% on HT-29Cl19A cells. This was the most rapid response at the lower concentration observed for this group.

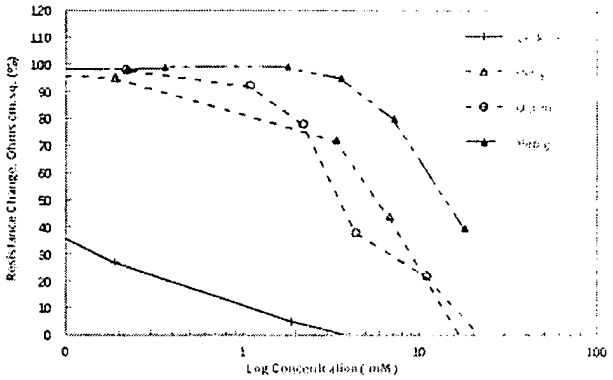


Figure 1. Change in resistance with concentration for T-84 cells. Transepithelial electrical resistance decreases across cell monolayers on addition of alkylglycoside. N = 3, SD < 5%. Dodec.m indicates dodecylmaltoside; Oct.g., octylglucoside; Oct.m., octylmaltoside; Hep.g., heptylglucoside.

Table 2. Alkylglycosides, Decrease in Resistance, and Cell Recovery - *Alkylglycosides are listed in three groups indicating suitability as permeability -enhancing agents. †Decrease in resistance of cells on application of alkylglycosides at concentrations of 0.01% and 0.1% in Ohm cm² (%) from 8 to 20 minutes. ‡Cell recovery indicated by increase in resistance in Ohm cm² (%) to original value within 18 hours after removal of the agent.

Alkylglycoside*	Decrease in Resistance†				Cell Recovery‡	
	T-84		HT-29		T-84	HT-29
No recovery	0.01%	0.1%	0.01%	0.1%		
Hexylmaltose	80		80		0	0
Nonylmaltose	80		80		0	0
Nonylglucoside	80		80		0	0
Tetradecylmaltose	80		80		0	0
Partial recovery						
Decylmaltoside		90		90	21 2	32 4
Tridecylmaltoside		90		90	29 2	32 3
Hexylglucoside		90		90	49 3	55 4
Decylglucoside		90		90	21 3	28 2
Dodecylglucoside		90		90	29 4	30 2
Extensive recovery						
Heptylglucoside		7 2		22 3	65 3	80 3
Octylglucoside		30 4		15 4	72 4	78 3
Octylmaltoside		20 4		35 4	73 3	85 4
Dodecylmaltoside		95 2		88 3	95 2	90 4

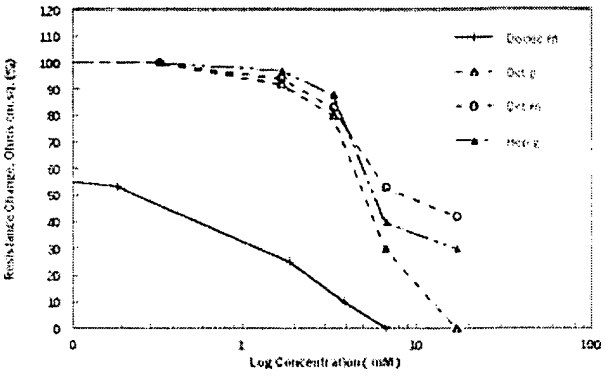


Figure 2. Change in resistance with concentration for HT-29Cl.19A cells. Transepithelial electrical resistance decreases across cell monolayer on addition of alkylglycoside. N = 3, SD < 5%. Dodec.m indicates dodecylmaltoside; Oct.g., octylglucoside; Oct.m., octylmaltoside; Hep.g., heptylglucoside.

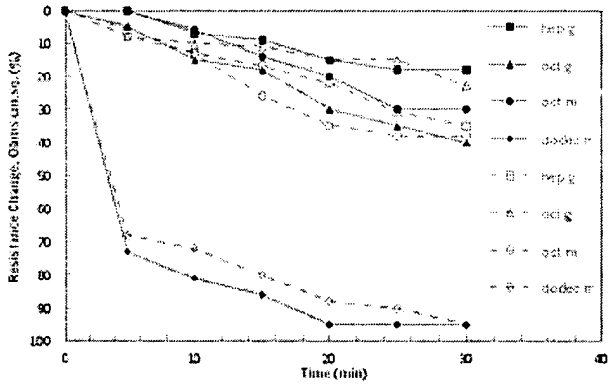


Figure 3. Change in resistance over time with accumulated additions of alkylglycosides to cell monolayers. N = 3, SD < 5%. Dodec.m indicates dodecylmaltoside; Oct.g., octylglucoside; Oct.m., octylmaltoside; Hep.g., heptylglucoside; s haded symbols, T-84 cells; open symbols, HT-29Cl.19A cells.

Addition of heptylglucoside, octylglucoside, octylmaltoside, and dodecylmaltoside to both cell lines was conducted over a total cell exposure of 30 minutes (Figure 3). Dodecylmaltoside was most effective in lowering resistance by around 90% in 20 minutes.

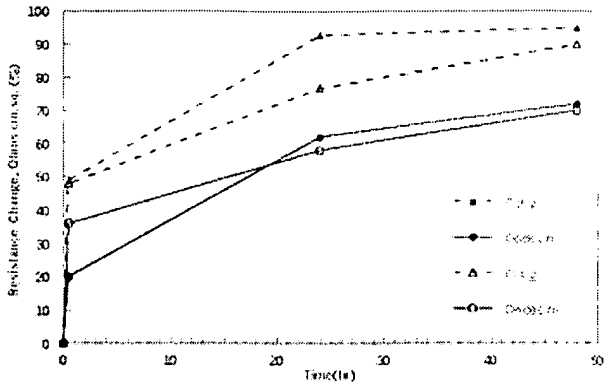


Figure 4. Recovery of cells shown by increase in resistance over time after repeated additions of alkylglycosides. Recovery time was recorded when the final application of alkylglycoside was removed after 48 hours. N = 3, SD < 5%. Dodec.m indicates dodecylmaltoside; Oct.g., octylglucoside; Oct.m., octylmaltoside; Hep.g., heptylglucoside; shaded symbols, T-84 cells; open symbols, HT-29Cl.19A cells.

After repeated applications of 2 alkylglycosides—octylglucoside and dodecylmaltoside for 6 hours—cells were observed to partially recover after 48 hours (Figure 4). With each application, resistance was lowered by around 30% in response to octylglucoside addition and 80% with dodecylmaltoside. Within 30 minutes of removal of the agent, both cell lines showed a rapid recovery of 20% to 50%, followed by increasing recovery over 2 hours. With complete removal of the agent after 48 hours, a more efficient recovery of around 90% was observed in cells treated with dodecylmaltoside , whereas octylglucoside-treated cells improved by around 70%.

Addition of the brilliant blue dye alone to T-84 cell monolayers caused no significant change in resistance across cells. It was transported only in the presence of dodecylmaltoside or octylglucoside, which was confirmed by spectrophotometric analysis (Figure 5). No difference in the amount transported by the agents was apparent for 30 minutes. After 90 minutes, dodecylmaltoside allowed transport of dye, resulting in a concentration of 9.2 g/mL and a concentration of octylglucoside of 7.6 g/mL. Resistance was decreased

by around 80% to 90% over the exposure period, whereas recovery of cells after removal of alkylglycoside plus dye was incomplete, amounting to around 5% after 30 minutes and less than 40% after 18 hours.

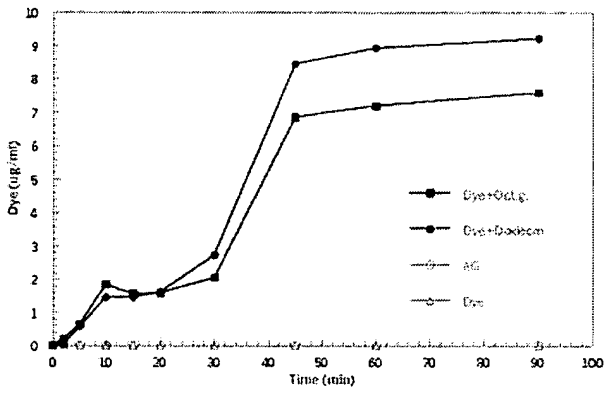


Figure 5. Amount of dye passing from apical to basolateral regions of T-84 cell monolayers as a function of time in the absence and presence of alkylglycoside. N = 3, SD < 5%. Dye indicates brilliant blue; Dodec.m, dodecylmaltoside; Oct.g., octylglucoside; Oct.m., octylmaltoside; Hep.g., heptylglucoside; AG, alkylglycoside.

DISCUSSION

Nine alkylglycosides proved unsuitable as permeability enhancers because of solubilization of cell membrane components to various degrees. Disruption of cell membranes at these concentrations appears to be sufficient to prevent reorganization of cell components.

Of the remaining alkylglycosides, 2 maltosides (octylmaltoside and dodecylmaltoside) and 2 glucosides (heptylglucoside and octylglucoside), allowed significant cell recovery after activating tight junctions (Table 2).

After repeated application of octylglucoside and dodecylmaltoside, cell recovery was incomplete after 48 hours (Figure 4). The behavior of cells in vitro should give some indication of how alkylglycosides might affect cells in clinical conditions. For meaningful practical use, cell recovery needs to be complete before further application of adjuvant that would require lower concentrations than those used currently.

The brilliant blue dye did not traverse T-84 cell membranes without the accompaniment of either octylglucoside or dodecylmaltoside (Figure 5). These alkylglycosides assisted the passage of dye and appeared to activate tight junctions, although some cell solubilization may have occurred. These results are encouraging, and partial cell recovery of only 40% in 18 hours may be the result of the combination of this particular dye and alkylglycoside. A correlation between increasing concentration and lowering resistance has been established (Figures 1 and 2, Table 2).

It seems unlikely that a relationship exists between the structures of these alkylglycosides and their effects. Activity appeared to be unrelated to the carbon chain length or the carbohydrate moiety of the alkylglycoside.

All alkylglycosides under consideration appear to increase fluidity of cell membranes to some degree, and although it was expected that the solubilizing capacity would increase with increasing alkyl chain length, this was not apparent. Nonionic surfactants produce a pronounced decrease in critical micelle concentration (cmc) with increase in hydrocarbon chain length, and there may be a relationship between the cmc of the alkylglycosides and their solubilizing effect [12].

Claude and Goodenough [13] proposed that resistance across cell monolayers is proportional to the number of strand elements found within tight junctions. It is possible that the various degrees of cell recovery after alkylglycoside application and removal are associated with the graduated regeneration of these strands coupled with extracellular calcium ions.

Partial recovery of cells indicates a more pronounced and lasting effect of the alkylglycosides and may indicate some solubilization of cell membranes. Advanced membrane disruption is suffered by cells that do not recover presumably because the alkylglycoside is irreversibly solubilizing membrane components.

Of the 13 alkylglycosides evaluated, only 2 were found to be suitable candidates for further use. Both dodecylmaltoside and octylglucoside show activity at low concentrations, causing a rapid fall in resistance and a short cell recovery time. These characteristics indicate negligible disruption of cell membranes, suggesting a possible tight junction mechanism.

In the presence of dodecylmaltoside or octylglycoside at low concentrations, the passage of a solute may be enhanced. In cases where drug transport is inefficient because of the compromise of intact drug, these alkylglycosides may help to maintain its therapeutic viability. This project has laid the foundation for further investigation into use of selected alkylglycosides in assisting drug transport across biological membranes.

ACKNOWLEDGEMENTS

The author would like to thank the Burroughs Wellcome Fund and the American Foundation for Pharmaceutical Education for support in the form of a New Investigations Grant from the American Association of Colleges of Pharmacy.

The author would also like to thank Paula Thompson, PharmD, for critical revision of this manuscript.

REFERENCES

1. Moses AC, Gordon GS, Carey MC, Flier JS. Insulin administered intranasally as an insulin-bile salt aerosol: effectiveness and reproducibility in normal and diabetic subjects. Diabetes. 1989:32;1040-1047.

2. Atchison JA, Grizzle WE, Pillion DJ. Colonic absorption of insulin: an in vitro and in vivo evaluation. J Pharmacol ExpTherap. 1989:248;567-572.

3. Chiou GCY, Chuang C, Chang MS. Reduction of blood glucose concentrations with insulin eye drops. Diabetes Care. 1988:11;750-751.

4. Saffron M, Kuwar C, Savariar C, Burham J, William F, Neckers D. A new approach to the oral administration of insulin and other peptide drugs. Science. 1986:233;1081-1084.

5. Dimitrijevic D, Shaw AJ, Florence AT. Effects of some non-ionic surfactants on transepithelial permeability in Caco-2 cells. J Pharm Pharmacol. 2000:52(2);157-162.

6. Swenson ES, Milisen WB, Curatolo W. Intestinal permeability enhancement: efficiency, acute local toxicity and reversibility. Pharm Res. 1994:11(8);1132-1142.

7. Gordon GS, Moses AC, Silver RD, Flier JS, Carey MG. Nasal absorption of insulin: enhancement by hydrophobic bile salts. Proc Natl Acad Sci U S A. 1985:82;7419-7423.

8. Pontiroli AE, Alberetto M, Pajetta E, Calderara A, Pozza G. Human insulin plus sodium glycocholate in a nasal spray formulation: improved bioavailability and effectiveness in normal subjects. Diabetes Metab. 1987:13;441-443.

9. Pillion DJ, Hosmer S, Meezan E. Dodecylmaltoside-mediated nasal and ocular absorption of lyspro-insulin: independence of surfactant action from multimer dissociation. Pharm Res. 1988:15(10);1637-1639.

10. Pillion DJ, Bartlett JD, Meezan E, Yang M, Crain RJ, Grizzle WE. Systemic absorption of insulin delivered topically to the eye. Invest Ophthalmol Vis Sci. 1991:32;3021-3027.

11. Murakami M, Kusanoi Y, Takada K, Muranishi S. Assessment of enhancing ability of medium-chain alkylsaccharides as new absorption enhancers in rat rectum. Int J Pharm. 1992:79;159-169.

12. Attwood D, Florence AT. Surfactant Systems: Their Chemistry, Pharmacy and Biology. London, UK: Chapman and Hall, 1983:88-90.

13. Claude P, Goodenough D. Fracture faces of zonulae occludens from "tight" and "leaky" epithelia. J Cell Biol. 1973:58;390-400.